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**Structural and Functional Analysis of Interhelical Interactions in the HIV-1 gp41 Envelope Glycoprotein by Alanine-Scanning Mutagenesis**

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Beamline(s): X12B

**Introduction**: Packing interactions between the central coiled-coil trimmer and the C-terminal helix of the gp41 ectodomain core are thought to be important determinants of HIV-1 entry and its inhibition. It is of fundamental importance to understand the structural and mechanistic basis of helical interactions in this core.

**Methods and Materials**: Rhombohedral crystals of G572A were grown from 10 mg $ml^{-1}$ peptide, 0.1 M sodium acetate (pH 5.2), 0.2 M ammonium sulfate, and 10% polyethylene glycol 4000. Rhombohedral crystals of R579A peptide were obtained from 20 mg $ml^{-1}$ peptide, 0.1 M sodium acetate (pH 4.5), 0.2 M ammonium sulfate, and 29% polyethylene glycol methyl ether 2000. Diffraction data were collected at 95K using a Quantum-4 CCD-based detector at the X12B Beamline of the National Synchrotron Light Source.

**Results**: The overall folds of G572A and R579A are very similar to that of the wild-type molecule. Both the Gly-572 and Arg-579 residues replaced in G572A and R579A, respectively, are in ***g*** positions of N34. Three C28 helices pack obliquely into the highly conserved hydrophobic grooves of the N34 coiled-coil trimmer in an antiparallel orientation, mainly through residues at the ***a*** and ***d*** positions of C28. On the surface of the N34 trimeric coiled coil, there are three prominent, symmetry-related cavities (~400 $Å^3$) that accommodate three hydrophobic residues from the abutting C28 helix: Trp 628, Trp 631, and Ile 635. The substituted Ala-572 side chains point into the triangular interhelical space between two N34 helices and a buttressing C28 helix. The favorable van der Waals interactions between the Ala-572 and Trp-631 side chains can strength interhelical packing and stabilize the trimmer-of-hairpins structure, as suggested by the large increase in $T_m$ of G572A relative to that of N34(L6)C28. In addition, the Gly-572-to-Ala substitution also leads to local structural rearrangements: the side chains of Trp-628 and Ile-635 deviate substantially. Overall, the methyl groups of the Ala-572 residues in G572A pack efficiently into the hydrophobic cavity of N34 and make a good C28 interaction.

**Conclusions**: In the present study, we have investigated the role of individual side chains at the nine ***e*** and ***g*** positions of the N34 coiled coil in conferring structural specificity and conformational stability to the gp41 core, and in determining the fusion potential of the envelope glycoprotein complex. Our alanine-scanning mutagenesis results show that the Leu-556, Leu-565, Val-570, Gly-572, and Arg-579 residues play a critical role in promoting HIV-1 membrane fusion, while the Val-549, Gln-551, Gln-563, and Gln-577 side-chains *per se* are not essential for membrane fusion activity. Our biophysical analysis reveals that alanine mutations in the fusion-defective L556A, L565A, and V570A envelope glycoproteins destabilizes the trimer-of-hairpins by a $T_m$ shift of ~6-14 °C. In contrast, the $T_m$'s of the four gp41 cores carrying the fusion-competent alanine mutations are comparable to or even higher than that of the wild-type molecule. In general, the stability of the trimmer-of-hairpins structure modulates the membrane fusion properties of the gp120-gp41 complex.

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